

Constitutive Heterochromatin Differentiation and Evolutionary Divergence of Karyotype in Oriental *Anopheles* (*Cellia*)¹

VISUT BAIMAI²

ABSTRACT: Analysis of the mitotic karyotype of two clusters of closely related species of oriental *Anopheles*, the *A. balabacensis* and *A. maculatus* complexes, has revealed interspecific differences in the amount and distribution of constitutive heterochromatin, particularly in sex chromosomes. Such a qualitative diagnosis of heterochromatin is useful in identification of these sibling species. The cytological evidence indicates a significant role of heterochromatin in chromosomal evolution of anopheline mosquitoes. The novel heterochromatin differentiation in sex chromosomes suggests an evolutionary role in the process of species divergence. Furthermore, extensive intraspecific variations of sex chromosome heterochromatin have been observed in natural populations of *A. dirus* A and B, while chromosomal rearrangement is very rare, if not absent. The gross heterochromatin variation may be correlated with variability in vectorial capacity, which may reflect its functional significance in coevolutionary processes.

CYTOLOGICAL VARIATION IS A general phenomenon of the eukaryotic genome. Chromosomal polymorphism usually persists in natural populations of higher organisms in two forms: chromosomal rearrangements and quantitative differences in constitutive heterochromatin. The former are well known in many groups of Diptera, and are believed to have some adaptive significance in different microenvironments (Dobzhansky 1970). The latter, on the other hand, although not uncommon in karyotypic evolution in many groups of eukaryotes (White 1973), is a form of polymorphism whose functional role and implications in species differentiation remain unclear (Chang and Carson 1985, review by John and Miklos 1979).

Detectable cytological differences are generally useful in studies of taxonomy and evolutionary relationships of many groups of

dipteran insects. Several authors working with *Drosophila* (Carson and Yoon 1982, Carson et al. 1970, Stalker 1972), Chironomidae (Martin et al. 1974, Newman 1977), and Simuliidae (Bedo 1977) have discussed phylogenetic relationships among closely related species groups through detailed investigation of chromosomal rearrangements. The application of this technique, based on comparatively fixed chromosome inversion and sex chromosome differences of unique arrays of chromosomal polymorphisms, is also useful in the recognition of cryptic species of anophelines (Coluzzi and Kitzmiller 1975, Green 1982, Green and Miles 1980, Lambert 1983, Saguna 1982, Subbarao et al. 1983).

Constitutive heterochromatin is a general feature of the chromosome complement in eukaryotic karyotypes. The pericentromeric heterochromatin can be readily demonstrated by C-band techniques (Arrighi and Hsu 1971). There have been numerous reports on the natural occurrence of quantitative heterochromatin variation in both plants and animals (e.g., Baverstock et al. 1982, John 1981, John and King 1977, 1983, Pathak et al. 1973, Patton and Sherwood 1982, Schweizer 1980, Weimarck 1975). Dif-

¹This investigation received financial support from the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases, the U.S. Army Component (AFRIMS), and Mahidol University Fund.

²Mahidol University, Department of Biology, Faculty of Science, Rama VI Road, Bangkok, Thailand 10400.

ferent degrees of staining intensity and different locations of constitutive heterochromatin apparently are due to different patterns of C-bands. This has been a useful cytotaxonomic technique (White 1978). Of particular interest, detectable heterochromatin differences may provide a useful criterion for identification of closely related species, for example, in *Drosophila* (Baimai and Ahearn 1978, Baimai et al. 1983). Recently, this C-band method for detecting heterochromatin differences together with new fluorescence techniques have been used successfully in separating certain cryptic species of *Anopheles* (Baimai et al. 1981, Gatti et al. 1977, Vasantha et al. 1982, Wibowo et al. 1984). Thus, heterochromatin variation in natural populations is of special interest as a model for examining its possible role in evolutionary divergence at the genomic level.

From the evolutionary genetics point of view, anopheline mosquitoes are one of the most interesting groups of the oriental fauna. Some of the *Anopheles* species groups play very important roles in transmission of human pathogens in the tropical region. Of the genus *Anopheles*, subgenera *Anopheles* and *Cellia* are the most predominant groups, each with some 200 known species (Harrison and Scanlon 1975, Rao 1984, Reid 1968). Some of these described species have received close attention for detailed investigation because they serve as important vectors for human malarial parasites. Naturally, much of the genetic studies of anopheline mosquitoes have been made mainly as applied field researches on relatively few species of medical importance (Coluzzi and Kitzmiller 1975, Kitzmiller 1976, White 1980). Little work has been done broadly in terms of evolutionary biology. Like all other dipterans, information on chromosomal polymorphism of anophelines is best known from studies of chromosomal rearrangements as seen in the polytene chromosomes prepared from larval salivary glands or ovarian nurse cells of half-gravid females (Coluzzi et al. 1979, Green 1982, Kitzmiller et al. 1973). In contrast, less emphasis has been made with regard to the analysis of metaphase karyotype of anophelines in spite of its small number of

chromosomes ($2n = 6$) with extensive heterochromatin variation. Kitzmiller (1976) listed a total of only 30 species of *Anopheles* of which metaphase karyotype descriptions or figures had been demonstrated. More recently, there have been some reports dealing with inter- and intraspecific variation of sex chromosome heterochromatin (Baimai et al. 1984a, Bonaccorsi et al. 1980, Gatti et al. 1977, Green et al. 1985, Vasantha et al. 1982, Wibowo et al. 1984).

Comparative studies on karyotype analysis of constitutive heterochromatin and chromosomal rearrangements thus offer a promising opportunity to explore the implications of natural chromosomal variation for a better understanding of the microevolutionary process of oriental anophelines. This paper summarizes the cytological observations in some *Anopheles* species groups that suggest the evolutionary role of heterochromatin differentiation and briefly outlines some of its implications in the epidemiology of human malarial parasites.

SERIES NEOMYZOMYIA

A focal point of attention is the *leucosphyrus* species group, belonging to the Neomyzomyia series of the subgenus *Cellia*, since most of its species members, especially the *Anopheles balabacensis* complex, are primary vectors of human malarial parasites in their particular areas of distribution. This species complex is exclusively a forest and foothills group, and is widely distributed throughout the oriental region ranging from India (Assam) through all countries in Southeast Asia, Taiwan, and the southern part of the Republic of China (Figure 1). Early systematic studies of this species complex showed a wide range of geographical and morphological variation (Colless 1956, Reid 1968). Recently recognized species of the *A. balabacensis* complex include *A. balabacensis*, sensu stricto from Sabah and the Philippines, *A. takasagoensis* from Taiwan, and *A. dirus* from Thailand (Peyton and Harrison 1979, 1980). The results of cytogenetic studies of these species showed that they

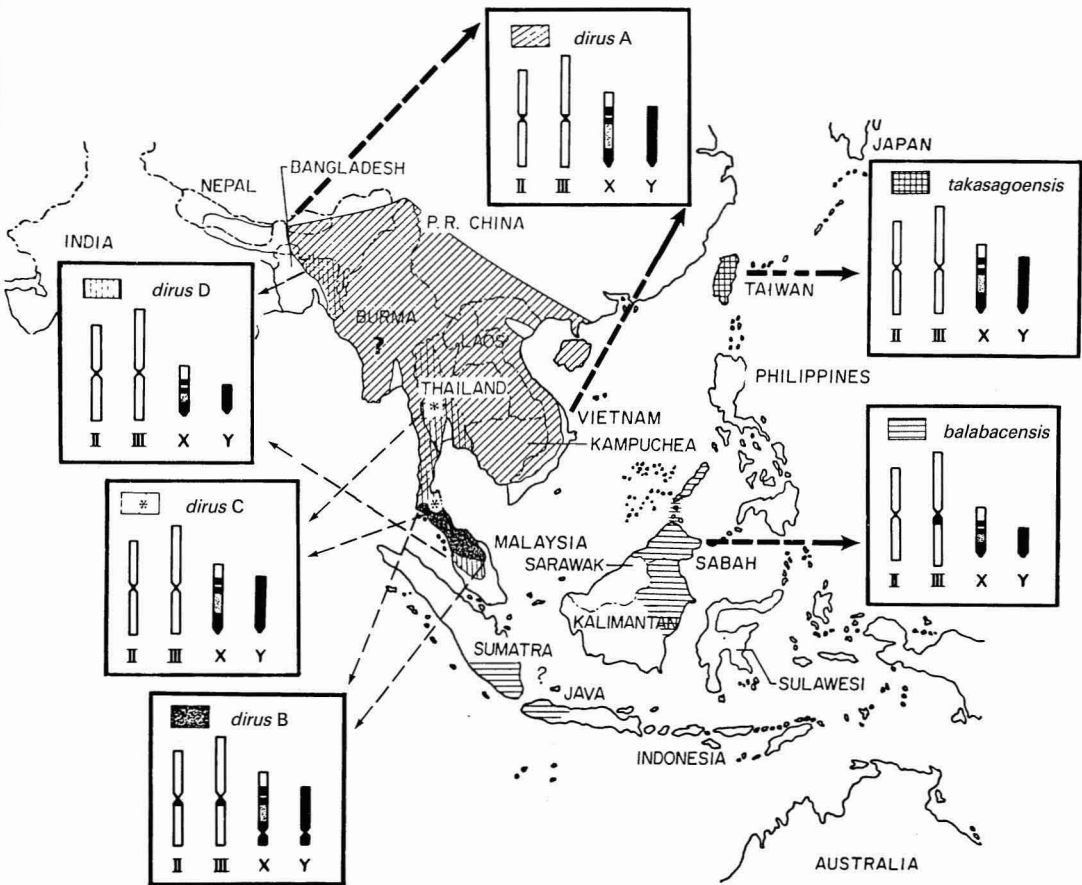


FIGURE 1. Distributions of the six species of the *Anopheles balabacensis* complex in Southeast Asia, depicted with diagrammatic representations of mitotic chromosomes using Giemsa staining. Heterochromatin is indicated in black or shaded.

were distinct biological species (Baimai et al. 1981, Hii 1982). However, Kanda et al. (1981, 1983) have suggested that the strains of “*A. balabacensis*” from Thailand, peninsular Malaysia, and Sabah represent three subspecies of this species complex. Detailed taxonomic studies (Peyton and Harrison, personal communication) together with cytogenetic analysis of natural populations of *A. dirus* (Baimai et al. 1984b, 1987) have revealed that it is actually a cluster of at least four closely related species provisionally designated as *dirus* A, B, C, and D. These siblings are only partially morphologically distinguishable, but they can be separated

easily by chromosomal analyses. In addition, species members of the *A. balabacensis* complex have different geographical distributions (Table 1, Figures 1, 2). *Anopheles balabacensis* is restricted to Sabah, East Kalimantan, and Palawan, while *A. takasagoensis* has been recorded only from Taiwan. Members within the taxon *A. dirus* have been found on the mainland of Southeast Asia. It is interesting to note that *A. dirus* D is relatively widespread in western Thailand, occurring in sympatry with its siblings. On the other hand, *A. dirus* A is common in central and northern Thailand, while *A. dirus* B is narrowly distributed in the area of the Thailand–Malaysia

TABLE 1

COLLECTING DATA FOR POPULATION SAMPLES AND MITOTIC SEX CHROMOSOME CONFIGURATIONS OF THE
A. balabacensis AND *A. maculatus* COMPLEXES

| SPECIES | LOCALITY (COLLECTION DATE, MONTH/YEAR) | SEX CHROMOSOME | |
|---------------------------------------|---|----------------|----|
| | | X | Y |
| <i>Anopheles balabacensis</i> complex | | | |
| <i>dirus</i> A | 1-CM(9/84), 2-LP(6/84), 3-PA(8/83), 4-PN(9/84), 5-LO(7/84), 6-KN(10/80), 7-NN(9/82), 8-PB(8/82), 9-TL(8/83), 10-CH(8/83), 11-RN(10/84), 16-SK(8/85) | t* | t* |
| <i>dirus</i> B | 12-PG(6/82), 14-PT(2/85), 15-TG(5/82) | a* | a* |
| <i>dirus</i> C | 6-KN(10/80), 13-SC(12/84), 14-PT(2/85) | t* | t |
| <i>dirus</i> D | 1-CM(9/84), 4-PN(9/84), 6-KN(10/80), 7-NN(9/82), 8-PB(8/82), 9-TL(8/83), 10-CH(8/83), 11-RN(10/84), 12-PG(6/82) | t* | t |
| <i>balabacensis</i> | SAB (4/84) | t* | t |
| <i>takasagoensis</i> | TAW† | t* | t |
| <i>Anopheles maculatus</i> complex | | | |
| <i>maculatus</i> A | 1-CM(9/84), 3-PA(8/83), 6-KN(10/82), 7-NN(9/82), 9-TL(8/83), 10-CH(8/83) | t | t |
| <i>maculatus</i> B | 1-CM(9/84), 7-NN(9/82), 9-TL(8/83), 10-CH(8/83) | sm* | a |
| <i>maculatus</i> C | 1-CM(9/84), 6-KN(10/82) | t | t |
| <i>maculatus</i> G | 9-TL(8/83) | sm* | sm |

NOTE: t = telocentric (rod shape); a = acrocentric; sm = submetacentric. See Figure 2 for locality numbers and abbreviations of collecting sites; SAB = Sabah, East Malaysia; TAW = Taiwan.

* Heterochromatin variation observed.

† Laboratory colony maintained at AFRIMS.

border (Figure 2). These two species are allopatric. Species C has a somewhat limited distribution in southern peninsular Thailand.

Analyses of larval mitotic chromosomes of the six members of the *Anopheles balabacensis* complex using conventional Giemsa staining methods (Baimai et al. 1981) and Hoechst 33258 fluorescent banding techniques (Wibowo et al. 1984) have revealed marked differences in the amount and distribution of constitutive heterochromatin both in sex chromosomes and at centromeric positions of autosome(s) (see Figures 1, 3). The metaphase karyotype ($2n = 6$) of all members of the *A. balabacensis* complex, except for *A. dirus* B, exhibits typical telocentric sex chromosomes of various sizes, which are clearly attributable to different amounts of heterochromatin in the vicinity of the centromere as well as qualitative differences of intercalary heterochromatin. Thus, the sex chromosomes of *A. dirus* D are relatively smaller than those of *A. dirus* A, C, and

takasagoensis, each of which shows X and Y chromosomes of roughly similar size. Nevertheless, *A. dirus* A, C, and *takasagoensis* display significantly different fluorescent banding patterns of major blocks of intercalary heterochromatin in the X and Y chromosomes, although they cannot be readily distinguishable by the Giemsa staining technique (compare Figures 1 and 3). The sex chromosomes of *A. dirus* B, on the other hand, are unique in having distinct acrocentric configurations. The short arm of these sex chromosomes is entirely heterochromatic, which is likely to be due to the addition of extra heterochromatin, although the possibility of pericentric inversion of the centromeric heterochromatin cannot be ruled out. Additionally, *A. dirus* B shows a considerable amount of centromeric heterochromatin in all autosomes. Thus, the apparent heterochromatin differentiation is most extensive in *A. dirus* B compared with the other members of the complex. While sex chromosomes of

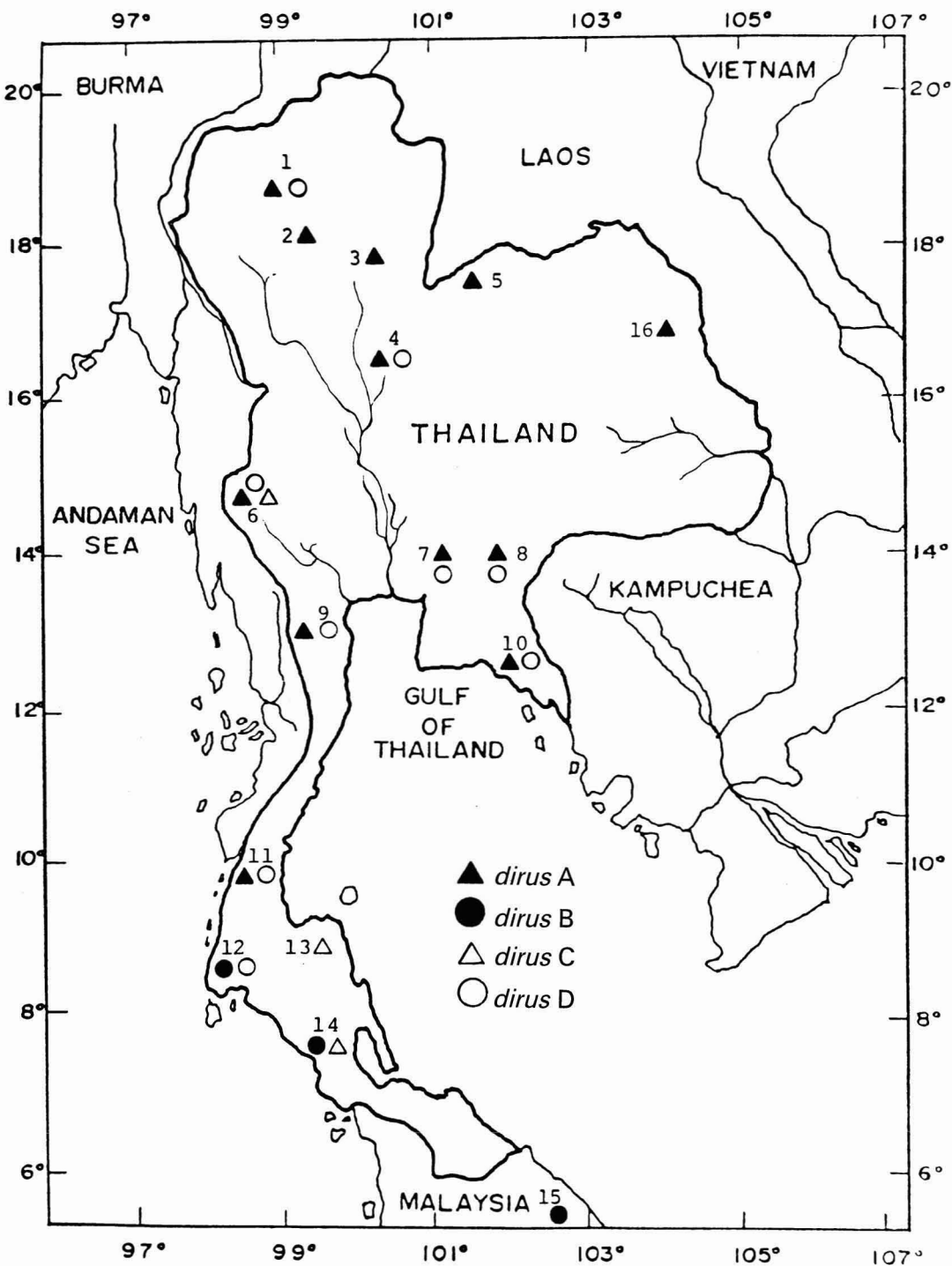


FIGURE 2. Map of Thailand and northern part of peninsular Malaysia showing collection localities of the four siblings within the taxon *Anopheles dirus* used for chromosome analyses. The localities (with abbreviations) of these sibling species and sample sizes (indicated in parentheses) are as follows: 1, Chiangmai-CM (A = 14, D = 6); 2, Lampang-LP (A = 6); 3, Phrae-PA (A = 15); 4, Pitsanulok-PN (A = 133, D = 15); 5, Loei-LO (A = 6); 6, Kanchanaburi-KN (A = 7, C = 9, D = 5); 7, Nakhon Nayok-NN (A = 4, D = 3); 8, Prachinburi-PB (A = 7, D = 5); 9, Petchaburi-TL (A = 17, D = 25); 10, Chantaburi-CH (A = 16, D = 12); 11, Ranong-RN (A = 2, D = 59); 12, Phangnga-PG (B = 2, D = 5); 13, Sichol-SC (C = 73); 14, Phatthalung-PT (B = 46, C = 2); 15, Trengganu, Malaysia-TG (B = 5); 16, Sakon Nakhon-SK (A = 52).

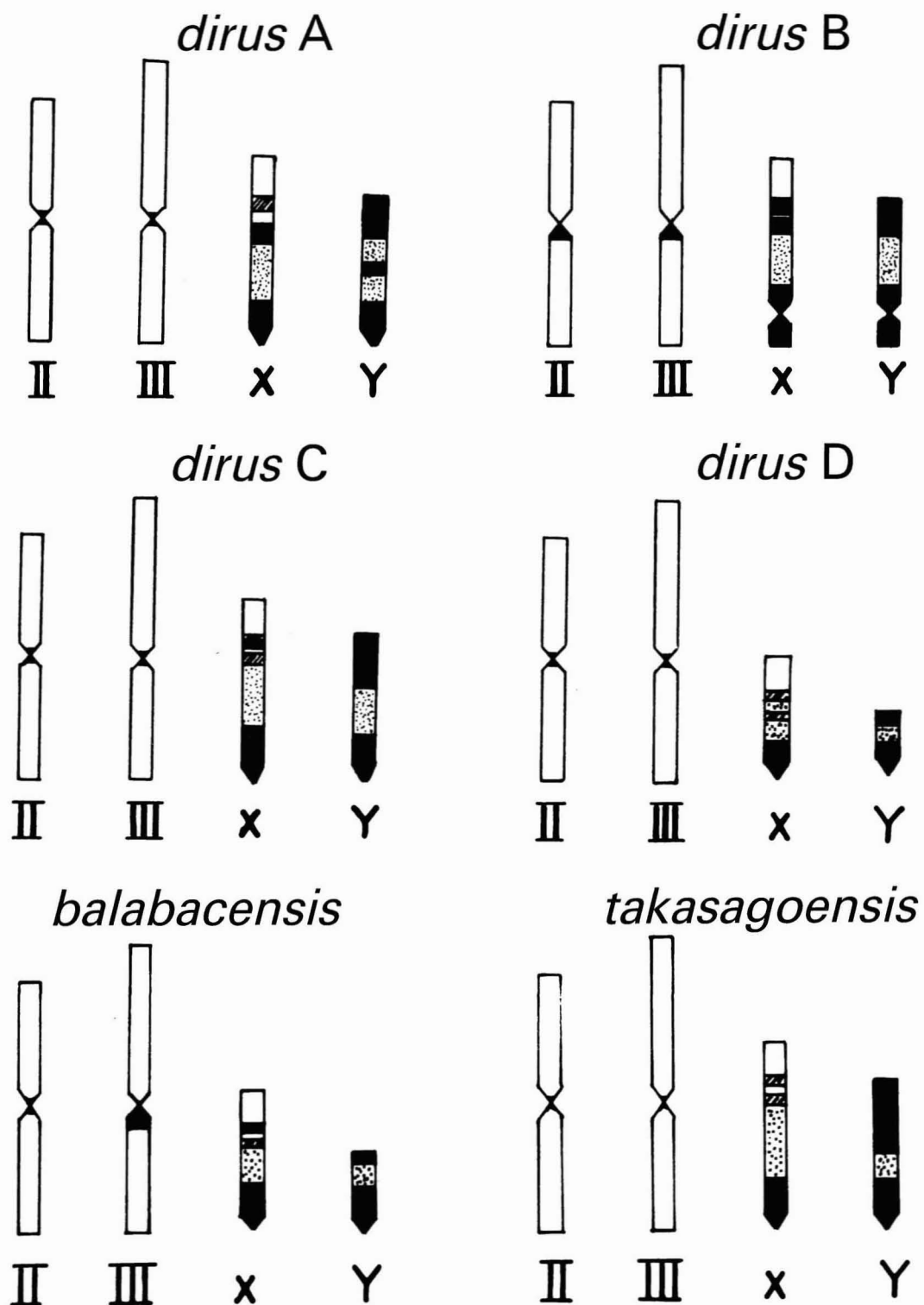


FIGURE 3. Diagrammatic representations of fluorescent banding patterns of mitotic karyotypes of the six species of the *Anopheles balabacensis* complex. Variable heterochromatic portion is indicated in black or shaded.

A. balabacensis appear to be similar to those of *A. dirus* D, these two species members do show significant differences in centromeric heterochromatin of autosome III (Figure 3). These two are, of course, allopatric species. The remaining four members of this species complex do not exhibit any detectable differences of centromeric heterochromatin of the autosomes. Such a gross chromosome difference with respect to constitutive heterochromatin is quite useful in species identification of this species complex and is routinely employed in our laboratory. Furthermore, natural population samples of all siblings within the taxon *A. dirus* in Thailand exhibit quantitative variation of centromeric heterochromatin of the X chromosome. To complicate matters further, *A. dirus* A and B show even more extensive heterochromatin variation in the vicinity of the centromere of both X and Y chromosomes (Baimai and Traipakvasin 1987, Baimai et al. 1984a). The evolutionary role and epidemiological significance of such an extensive heterochromatin variation remain unknown.

Data on polytene chromosome analysis of the *Anopheles balabacensis* complex is somewhat less extensive due to the difficulty of making reasonably good salivary gland chromosome preparations. The limited data available indicate that *A. dirus* D is relatively highly polymorphic for chromosomal rearrangements. At least six chromosome inversions have been observed distributed in five chromosome arms (two in arm 2R and one each in other arms) in Ranong populations. Species A shows a simple inversion in the X chromosome in some populations. On the contrary, no chromosomal inversion is recorded in *A. dirus* B and C from the Thailand population samples examined so far (Baimai, unpublished). Chromosomal rearrangement of *A. balabacensis* from Sabah is also rare, if not absent (Hii, personal communication), while chromosomal polymorphism in natural populations of *A. takasagoensis* from Taiwan is not known. Striking differences have been found in the free ends of X chromosomes of *A. dirus* A, B, and *balabacensis*, even though the general banding patterns of the other polytene chromosome arms were

almost identical (Hii 1982). In addition, *A. dirus* D shows a fixed paracentric inversion in the X chromosome that differs from the banding sequence of other species members (Baimai et al. 1987).

It may be noted that *Anopheles dirus* A and C are virtually homosequential species, showing almost identical banding patterns and nearly complete synapsis in F_1 hybrid polytene chromosome complement, except for the density of bands and sequences at the very tips of chromosome arms 2R, 2L, and the X chromosome (Baimai et al. 1987). Such a remarkable cytological difference is extremely useful for the unambiguous identification of these two genetic species, since they cannot be distinguished by mitotic sex chromosomes using conventional Giemsa methods, as mentioned above. This phenomenon of banding differences at the free ends of polytene chromosome arms has been observed in some closely related species of *Drosophila* (Hägele and Ranganath 1982), especially certain homosequential species of Hawaiian *Drosophila* (Ahearn and Baimai 1987).

Even though these observations tend to limit the use of mitotic karyotype analysis as a reliable guide to phylogenetic relationships, the cytological evidence presented here seems to suggest a significant role of heterochromatin in evolutionary divergence of the *Anopheles balabacensis* species complex. In the case of sympatric species, for instance, *A. dirus* A and D or *A. dirus* B and D, there has been no record of interspecific hybridization in nature. However, laboratory cross-mating experiments among these members were possible by artificial insemination, and produced variable degrees of reproductive isolation. Data from these hybridization experiments between *A. dirus* A and C indicate that they are the least reproductively isolated species compared to other member species (Baimai et al. 1984b, 1987). Cross-mating involving *A. dirus* A ♂ × C ♀ produced all fertile F_1 hybrids, while the reciprocal cross yielded fertile F_1 hybrid females but sterile F_1 males. All other combinations of interspecific crosses gave either sterile F_1 hybrid males or even sterile eggs, which reflected

high degrees of genetic incompatibility. These hybridization data seem to support the chromosomal observations mentioned earlier.

Usually, the general trend of chromosomal evolution of eukaryotes tends toward gain of heterochromatin (John and Miklos 1979). In this view, *Anopheles dirus* A may conceivably be considered a common ancestor of this cryptic species, since it has maintained the supposedly original types of X_1 and Y_1 chromosomes. Moreover, the extraordinary condition of gross DNA content at the free ends of chromosome X, arm 2R, and arm 2L of *A. dirus* C makes it possible to conceive that this species was directly derived from *A. dirus* A. If such is the case, *A. dirus* C might be more recently evolved from *A. dirus* A than the other two sibling species. The process of speciation may be accompanied by change and/or amplification of DNA sequences at the free ends of the three chromosome arms, probably facilitated by differentiation of (intercalary) heterochromatin of the sex chromosomes. These kinds of chromosomal differentiation may be associated with feeding and mating behavior. Indeed, field observations indicate that outdoor biting activity of *A. dirus* C occurs at a high level in the early hours of the night (6:30–7:30 PM), then decreases sharply, and is maintained at a very low level through the remainder of the night. On the contrary, outdoor biting activity of *A. dirus* A starts relatively later in the first half of the night, with a peak period around 8:00–9:00 PM, and gradually decreases toward the second half of the night. Such differences in feeding and perhaps mating behavior may significantly reflect an underlying mechanism for species differentiation of these very closely related species. Concerning this aspect, it has been demonstrated that some control factors in mating behavior of *Anopheles* mosquitoes are associated with the Y chromosome (Fraccaro et al. 1977). Moreover, data from detailed cytological and genetic analyses of the Y chromosome of *Drosophila melanogaster* suggest that fertility factors are located in the Y chromosome (Gatti and Pimpinelli 1983). If this phenomenon of fertility factors and

control elements for mating behavior holds true in general for insects, it is easy to visualize the effects of heterochromatin differentiation in the Y chromosome in association with sexual isolation giving rise to the speciation process of this sibling species complex. These findings seem parallel to the situation encountered in the *D. grimshawi-bostrychadisjuncta* complex of Hawaiian *Drosophila* (Ahearn and Baimai 1987, Baimai and Ahearn 1978). Further in-depth investigation may help elucidate the evolutionary significance of heterochromatin differentiation encountered in sibling species within the taxon *A. dirus*.

Cytogenetic evidence suggests that *Anopheles dirus* B and D are remote from *A. dirus* A. There is also some suggestion that *A. dirus* B and D are independently derived from a common ancestor, *A. dirus* A. This involves accumulation of heterochromatin in sex chromosomes and autosomes in the case of *A. dirus* B and fixing of a paracentric inversion in the X chromosome and heterochromatin differentiation in *A. dirus* D. The present evidence is not conclusive. Since *A. balabacensis* and *A. takasagoensis* are geographically isolated from each other and from all other members within the taxon *A. dirus*, it is not difficult to envisage that these two distinct biological species arose by allopatric speciation via genetic divergence expressed, perhaps, by the accompanying heterochromatin differentiation.

SERIES NEOCELLIA

The *Anopheles maculatus* species group of the Neocellia series of the subgenus *Cellia* is of special interest because it exhibits geographically morphological variation in correlation with variable degrees of vectorial capacity in different areas of its distribution (Reid 1968). Detailed cytogenetic studies of "*A. maculatus*" samples of Thailand populations revealed that this taxon is in fact a cryptic species comprised of at least four closely related species that occur in sympatry at some localities. These members of the *A. maculatus* complex are provisionally designated *macu-*

latus A, B, C, and G, all of which show different banding patterns in ovarian polytene chromosomes as well as quantitative differences of sex chromosome heterochromatin (Green and Baimai 1984, Green et al. 1985). Like the *A. balabacensis* complex, Giemsa C-banding of the metaphase karyotype of four members of the *A. maculatus* complex displays distinctive X and Y chromosome configurations. The telocentric Y chromosome of *A. maculatus* A is longer than that of *A. maculatus* C. On the other hand, the entirely heterochromatic Y chromosome of *A. maculatus* B is obviously acrocentric compared with the extraordinarily large submetacentric Y configuration of *A. maculatus* G (see Green et al. 1985). The X chromosome of both *A. maculatus* A and C shows telocentric shape and similar size, with about two-fifths of the chromosome length at the distal end being euchromatic. In contrast, the X chromosome of *A. maculatus* B and G is clearly submetacentric, of which approximately two-thirds of the short arm at the distal region is euchromatic while the long arm is totally heterochromatic. Intraspecific heterochromatin variation in the X chromosome of *A. maculatus* B and G has been observed in natural samples (Baimai, unpublished). Therefore, apart from interspecific differences in chromosomal rearrangements, distinctive sex chromosome heterochromatin is also a species-specific cytological character of these members within the taxon *A. maculatus*. Like the *A. balabacensis* complex, trends of karyotypic evolution by members of the *A. maculatus* complex apparently are due to accumulation of sex chromosome heterochromatin. Furthermore, members of these four genetic species show morphological differences in certain stages of development including egg raft, pupal skin, and adult morphology. A formal taxonomic study of the *A. maculatus* group has been made by Rattanarithikul and Green (1986). Interestingly enough, populations of "maculatus B" in southern peninsular Thailand show significantly different frequencies of some chromosomal rearrangements from the central and northern populations. Such unique polytene banding patterns of southern popula-

tions of "maculatus B" may reflect a different form, or even subspecies, which is provisionally designated form E. Thus, form E is the only representative form of *maculatus* B in southern Thailand, where it is known as one of the vectors of human malarial parasites. Other members of the *A. maculatus* complex are not considered as vectors in Thailand. It is not known whether such cytological differences are correlated with variation in vectorial capacities among members of this species complex.

Field data are being gathered on the mitotic karyotype of inter- and intraspecific variation of sex chromosome heterochromatin of other species groups within the series *Anopheles annularis* and others of the oriental *Anopheles* (Baimai, unpublished). The dramatic evidence available so far seems to suggest that heterochromatin differentiation often plays an important role in karyotypic evolution in anopheline mosquitoes, at least in the oriental region.

EVOLUTIONARY ROLE OF CONSTITUTIVE HETEROCHROMATIN

Although the formulation of models for speciation in relation to heterochromatin differentiation is inevitably in the realm of speculation, the foregoing cytogenetic data indicate some implications of heterochromatin in the phylogenetic affinity and consequently the evolutionary divergence of these and other cryptic species of *Anopheles* in Southeast Asia.

Cytological differences may not exist in some closely related species, since cytological change does not appear to be an absolute prerequisite for speciation (Carson 1982a, Chang and Carson 1985). For example, several members of homosequential species groups of Hawaiian *Drosophila* are primarily recognized by morphological and behavioral differences (Carson 1982b, Carson and Kanehiro 1976, Val 1977). In contrast, in many taxa, cytological differences by virtue of either chromosomal changes (e.g., gene rearrangements, fusions, translocations) or heterochromatin variation, or both, occur between

closely related species or sibling species groups that are virtually similar in gross morphology. Such chromosomal changes are often thought to be involved, to some degree, in species formation (White 1978). A considerable number of animals, particularly insects, are assigned to certain phylogenetic relationships of species groups on the basis of chromosome differences and interspecific mating ability (Dobzhansky 1970, White 1973). The existing knowledge in karyotypic evolution comes mainly from detailed investigation of natural populations of animals, for instance, in *Drosophila* (e.g., Baimai et al. 1983, Clayton 1968, 1969, Patterson and Stone 1952, Yoon and Richardson 1978), stick insects (Craddock 1974), ants (Imai et al. 1977), grasshoppers (White 1974), and some groups of mammals (Baverstock et al. 1977, Mascarello and Hsu 1976, Pathak et al. 1973, Patton and Sherwood 1982). Data on karyotype analysis of different taxonomic groups of animals suggest a possible role of constitutive heterochromatin in the process of evolutionary divergence.

Increasing cytogenetic evidence has led to some new insights into the evolutionary biology of *Anopheles* that may afford answers to the possible relationships among closely related species groups and probable modes of speciation (Kitzmiller 1976). Further, recent recognition of cryptic species in some taxa of the oriental *Anopheles* provides a better understanding of mechanisms underlying the speciation processes of these medically important mosquitoes (Baimai et al. 1981, Green and Baimai 1984, Green et al. 1985, Hii 1982, Subbarao et al. 1983). Cytological differences in the karyotype and salivary gland polytene chromosomes of some species of Culicidae have been described by several workers (for a review, see Kitzmiller 1967, 1976). As exemplified in this paper, a striking feature of cytological variation of the oriental *Anopheles* is the wide range of chromosomal rearrangements and sex chromosome evolution via variable degrees of heterochromatin differentiation. Thus, cytological differences both in polytene chromosome inversions and in mitotic sex chromosome heterochromatin are conspicuous among the four species members of the *A. maculatus*

complex. In contrast, all species members of the *A. balabacensis* complex are generally homosequential, but they are strikingly different in the amount and distribution of constitutive heterochromatin in sex chromosomes and the centromeric autosomes. These metaphase karyotype differences may be accounted for by gain of heterochromatin, which is a common phenomenon in chromosome evolution (John and Miklos 1979). This kind of cytological difference is comparable to the situation of some homosequential species of the picture-winged Hawaiian *Drosophila*, (Ahearn and Baimai 1987, Baimai and Ahearn 1978, Clayton 1969). Cytogenetic evidence in these species groups and others of *Anopheles* examined so far suggests that sex chromosomes are particularly prone to the accumulation of extra heterochromatin, ranging from noticeably small, to moderately and extremely large amounts. The mechanism for the increased amount of heterochromatin is not fully understood. It may be the corollary of different phenomena, e.g., unequal crossing over and chromosomal breakage (review by John and Miklos 1979). Thus, heterochromatin differentiation may be generally considered as a recurrent feature of chromosome changes, which presumably serve as a medium for genetic divergence and subsequent evolutionary process.

Earlier, it was thought that heterochromatin was genetically inert and hence had no effect on the biology and viability of higher organisms (review by Brown 1966). However, biochemical evidence suggests that heterochromatin is correlated with highly repetitive DNA sequences or satellite DNA arranged tandemly in the vast majority of the genome (Britten and Kohne 1968, Peacock et al. 1977). The repeated DNA sequence of chromatin represents noncoding DNA, and consequently has no direct function or immediate phenotypic benefit. The presence or absence of a highly repetitive DNA sequence or heterochromatin does not seem to exert direct genetic effects similar to the unique DNA sequence associated with the addition or deficiency of euchromatin. Nonetheless, some repetitive DNA sequences may serve a regulatory function upon the unique DNA sequences (Britten and Davidson 1969, Hilliker and Appels

1980). Repetitive noncoding DNA also may show much faster sequence divergence than the unique DNA sequence of euchromatin (Macgregor et al. 1976). It has been suggested that species differentiation may involve some changes in the gene regulatory system besides mutation of the coding DNA (Britten and Davidson 1969). From this point of view, heterochromatin may be envisaged as a potential source of evolutionary change in the regulatory context. Hence, instead of using the term "genetically inert" for a highly repetitive DNA sequence or heterochromatin in the sense of selfish, symbiotic, or ignorant parasitic DNA (e.g., Doolittle and Sapienza 1980, Orgel and Crick 1980), we may consider the term "dynamic element" of the genome. Several investigators have evoked an evolutionary role of heterochromatin in attempting to explain the common phenomenon of heterochromatin differentiation (Dover et al. 1981, Nagl and Capesius 1977). As yet, there has been no compelling evidence supporting these views.

The karyotypic differences among members of the *Anopheles dirus* and *A. maculatus* species complexes are due mainly to the changes in quantity and/or quality of sex chromosome heterochromatin. The direction of chromosome changes tends, in most cases, toward the acquisition of extra heterochromatin, which can be comparatively determined by Giemsa banding techniques. Thus, almost all Thailand populations of these species subgroups harbor large amounts of heterochromatin variation in sex chromosomes, which may reflect, to some degree, the different interspecific nature of regulatory gene systems. The critical problems revolve around how consistent this kind of heterochromatin differentiation is, and whether any differentiation is based on persistent genetic divergence. If this type of heterochromatin differentiation is to play any significant role in influencing levels of genetic divergence within populations of these mosquitoes, it should be a repeatable phenomenon both within populations of a species over several seasons and between populations of different species that have experienced the same set of selective pressures of the genome. In any event, the present data seem to suggest the

preeminence of sex chromosome heterochromatin in the evolutionary divergence of these closely related species groups, although they do not deal explicitly with the postulated correlation. Further investigation into the dynamic element of heterochromatin and its evolutionary significance remains intriguing and challenging.

HETEROCHROMATIN VARIATION AND EPIDEMIOLOGICAL IMPLICATIONS

It seems clear that heterochromatin is involved in karyotypic evolution in many groups of organisms, but the functional role of heterochromatin has not been established. By and large, heterochromatin does not interfere with the normal function and development of the organisms containing it, but whether it attributes some advantages to the organism is still obscure. There is an increasing awareness of the possibility that heterochromatin may exert some functional role in the internal environment, where it behaves as a not-too-harmful "parasite" within the host cell. In this view, one may speculate that the extra ("parasitic") heterochromatin of the insect vectors might exercise its dynamic role in interacting favorably with the true parasites, e.g., *Plasmodium* transmitted by the mosquito hosts. Thus, heterochromatin differentiation may play an important role in coevolutionary processes. Such speculation can be tested. In the *Anopheles gambiae* complex, differences in constitutive heterochromatin in the sex chromosomes may be related to different responses in vectorial capacity of two closely related species, *A. gambiae* and *A. arabiensis* (Bonaccorsi et al. 1980). From this viewpoint, it is suggested that a serious effort should be made to determine whether there is any correlation between heterochromatin differences and variable degrees of vectorial capacity, both within a species and between sibling species of potentially effective vectors, e.g., within the *A. dirus* and *A. maculatus* complexes. Both variable effects, susceptibility to malarial parasites and heterochromatin, of the vectors may be acting in concert in nature. In this respect, preliminary laboratory tests show that dif-

ferent members of these two *Anopheles* species groups display significant differences in susceptibility (R. G. Andre, personal communication). If heterochromatin variations in the sex chromosomes affect the genetic basis of susceptibility, then the development of strategies for vector control measures becomes critically important. It is not difficult to visualize how the sex chromosome heterochromatin might play an important role in reproductive isolation and, subsequently, species differentiation in some anopheline mosquitoes (Bonaccorsi et al. 1980, Fraccaro et al. 1977). Yet such a deductive interpretation is merely circumstantial and awaits suitable experimental tests.

Genetic changes ranging from point mutations through chromosomal rearrangement and including the load of highly repetitive DNA sequences are principally raw materials for evolutionary divergence. Studies of the latter should offer some insights pertaining to the evolutionary role of sex chromosome heterochromatin in speciation processes. The heterochromatin differentiation in sex chromosomes of the *Anopheles* species groups is an appropriate candidate for this approach. Although it may seem naive, this is an excellent opportunity to put evolutionary genetics into the field of applied research.

ACKNOWLEDGMENTS

I wish to thank H. L. Carson and W. W. M. Steiner for their useful comments on the manuscript. I also thank J. Hii for providing mosquito samples from Sabah. The expert technical assistance of A. Traipakvasin, U. Kijchalao, and K. Vejsanit is greatly appreciated.

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